

EFFECT OF SKELETAL UNLOADING ON BONE FORMATION

FINAL REPORT

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I. Description of Research.

The long-range goal of our research program is to understand the effects of gravity on skeletal development and bone metabolism. Our present objectives are to define the effects of gravity or mechanical stress on bone formation and resorption, and to determine the mechanisms (hormonal or paracrine) by which mechanical stress is coupled to bone cell activity.

Bone is a dynamic, living tissue. It continually is undergoing change, or remodeling, which involves a delicate balance between bone formation and bone resorption. This balance is influenced by systemic hormones such as parathyroid hormone, glucocorticoid hormones, growth hormone, and the vitamin D metabolites, as well as local factors such as blood flow, neuromuscular activity, and mechanical stress. Recently, a number of cytokines have been observed to stimulate or inhibit bone formation and resorption. Some of these cytokines, such as insulin-like growth factor-1 (IGF-1), transforming growth factors, and fibroblast growth factors, have been identified in bone. IGF-1 production by bone appears to be regulated by growth hormone. Such cytokines may participate in coupling mechanical stress to bone cell activity, or the activity of one type of cell to activity of another type of cell, for example, osteoblast activity to osteoclast activity. Our research over the past year was directed toward elucidation of the role that the systemic hormones play in coupling the mechanical stress of weight bearing to the cellular response of bone formation as well as beginning the assessment of the role of locally produced cytokines such as IGF-1.

We have continued to use the tail suspension model to produce skeletal unloading of the hind limbs of a growing rat. Bone formation was assessed by histomorphometry, ^{45}Ca and ^3H -proline incorporation, as well as change in fat-free weight and calcium content. Bone maturation was assessed by separating different fractions of powdered bone using toluene:bromoform density gradient centrifugation and evaluating the fractions for bone weight, total calcium, and ^{45}Ca and ^3H -proline incorporation. The effect of the active vitamin D metabolite $1,25(\text{OH})_2\text{D}$ on bone formation and mineralization was determined by infusing the $1,25(\text{OH})_2\text{D}$ into the rats with osmotic minipumps. Similar studies have been performed with growth hormone. The role of glucocorticoid hormones in mediating changes in bone formation with skeletal unloading was determined using surgical ablation techniques. To initiate studies of locally produced cytokines, we established cultures of mouse calvarial cells and long bone chondrocytes and plan to use such cultures to assess directly the effects of cytokines on bone cell function.

II. Accomplishments

1. Demonstrated that high physiological doses of $1,25(\text{OH})_2\text{D}$ cause a mineralization defect as detected by density gradient analysis.
2. Demonstrated that hindlimb elevation blunts the ability of growth hormone infusion to sustain growth of bone (tibia) in hypophysectomized rats.
3. Demonstrated a decrease in somatomedin C (IGF-1) concentration in the tibial growth plates from hindlimb elevated animals compared to pair fed controls.
4. Demonstrated that hindlimb elevation does not result in a change in serum corticosterone level or its Circadian rhythm.

5. Demonstrated that adrenalectomy does not prevent the inhibition of bone growth that occurs in the tibiae of the hindlimb elevated rat. Orchiectomy in combination with adrenalectomy was more effective than adrenalectomy alone in eliminating corticosterone production in the rats. This combination inhibited bone formation to such a degree that the additional effect of hindlimb elevation could not be discerned.
6. Demonstrated that bone formation is accelerated when rats are allowed to recover after hindlimb elevation such that the deficit in bone mass is nearly completely reversed in two weeks.
7. Demonstrated that fetal osteoblast cultures can be grown on collagen coated beads in the NASA Bioreactor. Under these conditions the cells lay down a collagen matrix and produce alkaline phosphatase.

III. Significance

Our current hypothesis is that the inhibition of bone formation that occurs transiently after skeletal unloading is due to a combination of systemic factors such as $1,25(\text{OH})_2\text{D}$ and locally produced factors such as somatomedin C (IGF-1). Our observation that $1,25(\text{OH})_2\text{D}$ infusion can lead to a mineralization defect encourages us to explore factors in bone under the control of $1,25(\text{OH})_2\text{D}$ that regulate mineralization. Osteocalcin is the most obvious candidate. This bone matrix protein is increased by $1,25(\text{OH})_2\text{D}$, and has been postulated to regulate bone crystal formation. In previous studies we have demonstrated that the concentration of osteocalcin in bone and blood falls during hindlimb elevation.

The decrease in somatomedin C levels in the growth plates of unweighted tibiae combined with the inability of growth hormone to reverse the inhibition of bone formation caused by unweighting suggests that the unloaded bone may have an abnormal response to growth hormone. This could be the key to understanding why bone formation is inhibited by unloading, since somatomedin C stimulates bone formation and its production by bone is thought to be under growth hormone control.

Our results indicating that adrenalectomy does not protect against the inhibition of bone formation by hindlimb elevation combined with our observations that corticosterone production is not increased by hindlimb elevation indicates that increased glucocorticoid production is not the reason bone formation is inhibited by hindlimb elevation.

The preliminary studies with osteoblasts in the Bioreactor point to future flight and ground based opportunities to assess the effects of gravity on bone cell function.

IV. Proposed projects for the coming year

1. Assess the role of growth hormone, IGF-1, and IGF-2 infusion on tibial bone formation in hypophysectomized rats either hindlimb elevated or pair fed. This will permit us to test directly the ability of the somatomedins (IGF-1 and IGF-2) to prevent the transient decrease in bone formation during unweighting.
2. Determine the effect of growth hormone, IGF-1, and IGF-2 on collagen production, alkaline phosphatase activity, osteocalcin production, and cell proliferation by fetal rat osteoblasts in vitro.

3. Determine the interaction of $1,25(\text{OH})_2\text{D}$ and growth hormone or IGF-1 and IGF-2 on the above functions of fetal rat osteoblasts.
4. Isolate bone cells and chondrocytes from the tibia of hindlimb unweighted and pair fed rats to determine whether they differ in their response to $1,25(\text{OH})_2\text{D}$, growth hormone, IGF-1, or IGF-2.

V. Publications:

1. Halloran BP, Bikle DD, Cone C, Morey-Holton E 1988 Glucocorticoids and the inhibition of bone formation induced by skeletal unloading. *Am J Physiol* E875-E879.
2. Halloran BP, Bikle DD, Castro M, Gee E 1988 Isotope labelling affects $1,25$ dihydroxyvitamin D metabolism. *Biochemistry* 28: 1278-1281.
3. Globus RK, Bikle DD, Morey-Holton E 1986 The temporal response of bone to unloading. *Endocrinology* 118:733-42.
4. Globus RK, Bikle DD, Halloran BP, Morey-Holton E 1986 Skeletal response to dietary calcium in a rat model simulating weightlessness. *J Bone Min Res* 1:191-7.
5. Halloran BP, Bikle DD, Wronski TJ, Globus RK, Levens MJ, Morey-Holton E 1986 The role of $1,25$ -dihydroxyvitamin D in the inhibition of bone formation induced by skeletal unloading. *Endocrinology* 118:948-54.
6. Halloran BP, Bikle DD, Levens MJ, Castro ME, Globus RK, Holton E 1986 Chronic $1,25$ -dihydroxyvitamin D_3 administration in the rat reduces the serum concentration of 25 -hydroxyvitamin D by increasing metabolic clearance rate. *J Clin Invest* 78:622-8.
7. Wronski TJ, Halloran BP, Bikle DD, Globus RK, Morey-Holton ER 1986 Chronic administration of $1,25$ -dihydroxyvitamin D_3 : increased bone but impaired mineralization. *Endocrinology* 119:2580-5.
8. Bikle DD, Halloran BP, Cone CM, Globus RK, Morey Holton E 1987 The effects of simulated weightlessness on bone maturation. *Endocrinology* 120:678-84.
9. Sessions ND, Halloran BP, Bikle DD, Wronski TJ, Cone CM, Morey-Holton E 1989 Bone response to normal weightbearing after a period of skeletal unloading. *Am J Physiol* 257:E606-610
10. Patterson-Buckendahl P, Globus RK, Bikle DD, Cone CE, Morey-Holton E 1989 Effect of simulated weightlessness on rat osteocalcin and bone calcium. *Amer J Physiol* 257:R1103-9, 1989.